UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/796,323	03/09/2004	Kyle B. Cole	3649.1	2108

22886 7590 04/17/2006

AFFYMETRIX, INC
ATTN: CHIEF IP COUNSEL, LEGAL DEPT.
3420 CENTRAL EXPRESSWAY
SANTA CLARA, CA 95051

EXAMINER	
MUMMERT, STEPHANIE KANE	
ART UNIT	**PAPER NUMBER**
1637	

DATE MAILED: 04/17/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/796,323	COLE ET AL.	
	Examiner	Art Unit	
	Stephanie K. Mummert	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *28 February 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-37* is/are pending in the application.

4a) Of the above claim(s) *33-37* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) _____ is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *6/24/05*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

1. Applicant's election without traverse of Group 1, claims 1-32 in the reply filed on February 28, 2005 is acknowledged.

Claims 33-37 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on February 28, 2006.

Claims 1-32 are pending and will be examined.

Information Disclosure Statement

2. The information disclosure statement (IDS) submitted on 6/24/05 was filed in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

Reference A21 on the IDS was not considered because the document number appears to be a typographical error as no PgPub documents were retrievable using the document number included.

Double Patenting

3. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re*

Goodman, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claims 1-6, 14, 16-18, 22-23, 25 and 31 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-4, 6, 20 and 24 of copending Application No. 10/951,983 ('983 herein) in view of McCarthy et al. (US PgPub 2004/0067559; April 2004). Although the conflicting claims are not identical, they are not patentably distinct from each other because the claims of the instant application and the copending application are nearly identical in the components/reagents and recited method steps claimed within the copending applications.

The difference(s) between the claims of the instant application and the '983 application is primarily seen in the different manner in which the limitations of the related methods are claimed. For example, in the instant application, the method of claim 1 includes steps directed towards the synthesis of cDNA in the presence of dUTP, while the copending application refers generally to synthesizing cDNA in the presence of a modified DNA precursor in claim 1, with dependent claim 2, limiting the precursor to dUTP.

This same issue arises for other limitations within claim 1 of the '983 application which are found in dependent limitations in the instant application. For example, claim 1 of '983 application claims a step of cleaving the cDNA at abasic sites followed by labeling the fragments

with biotin and hybridizing the fragments to a microarray of probes. These same limitations are claimed in the instant application, but these limitations are claimed in dependent claims 16-17 where the fragments are labeled and may be labeled with biotin, and claims 22-23 and 31 where the fragments are labeled and hybridized to an array of probes.

The major difference between the claims of the copending application and the instant application lies in the final step of the method, where the free 3'OH is labeled using a terminal transferase in the copending application, while the free 3'OH is extended by a strand displacing polymerase in the method of the instant application. It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have modified the technique of cDNA synthesis, fragmentation, and labeling taught by Blume to include the step taught by McCarthy, where the free 3'OH termini are extended using a strand displacing polymerase. As taught by McCarthy, "The method according to the invention provides a means of generating multiple copies of discrete single stranded primers downstream of an initiating primer. This offers exceptional specificity for detection purposes, as the discrete downstream primers can only be generated if the target template nucleic acid is present" (p. 5, paragraph 71). Given the benefit of specificity in detection provided by the method taught by McCarthy, one of ordinary skill in the art at the time the invention was made would have been motivated to substitute the step of extending the free 3'OH using a strand displacing polymerase for the step of labeling using a terminal transferase as taught by Blume with a reasonable expectation for success to achieve improved specificity for detection.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Claim Rejections - 35 USC § 103

4. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

5. Claims 1-6, 9, 12-18, 22-24 and 31 are rejected under 35 U.S.C. 103(a) as being unpatentable over Blume et al. (US PgPub 2005/0123956; June 2005) in view of McCarthy et al. (US PgPub 2004/0067559; April 2004). Blume teaches methods and compositions for fragmenting nucleic acid samples (Abstract).

The applied reference has a common assignee with the instant application. Based upon the earlier effective U.S. filing date of the reference, it constitutes prior art only under 35 U.S.C. 102(e). This rejection under 35 U.S.C. 103(a) might be overcome by: (1) a showing under 37 CFR 1.132 that any invention disclosed but not claimed in the reference was derived from the inventor of this application and is thus not an invention "by another"; (2) a showing of a date of invention for the claimed subject matter of the application which corresponds to subject matter disclosed but not claimed in the reference, prior to the effective U.S. filing date of the reference under 37 CFR 1.131; or (3) an oath or declaration under 37 CFR 1.130 stating that the application and reference are currently owned by the same party and that the inventor named in the application is the prior inventor under 35 U.S.C. 104, together with a terminal disclaimer in accordance with 37 CFR 1.321(c). This rejection might also be overcome by showing that the reference is disqualified under 35 U.S.C. 103(c) as prior art in a rejection under 35 U.S.C. 103(a). See MPEP § 706.02(l)(1) and § 706.02(l)(2).

With regard to claim 1, Blume teaches a method of amplification of a nucleic acid sample comprising:

a) obtaining a nucleic acid sample (p. 8, paragraph 86, where RNA transcript samples are used as templates);

b) contacting the nucleic acid sample with the appropriate reagents to synthesize a first strand cDNA (p. 8, paragraph 86, where the transcripts are used as templates for reverse transcription);

c) synthesizing a second strand cDNA in a reaction mixture comprising dUTP (p. 8, paragraph 86, where dUTP is incorporated into cDNA during first or second strand cDNA synthesis, see Figures 1 and 2);

d) nicking the second strand cDNA at one or more portions where dUTP was incorporated to generate one or more nicks (p. 8, paragraph 86, where uracil DNA glycosylase recognizes uracil in DNA and the uracil is removed, followed by treatment with an endonuclease such as endonuclease IV or V, leaving a 3'OH free); and

e) extending the second strand cDNA from the one or more nicks in a reaction mixture comprising dUTP and a DNA polymerase with a strand displacing activity, wherein downstream fragments of the second strand cDNA are displaced (p. 9, paragraph 105 or 122, where the labeling reaction is described p. 2, paragraph 21, legend to Figure 1, where the free 3' OH is labeled by polymerization with terminal transferase).

With regard to claim 2, Blume teaches an embodiment of claim 1 wherein steps d) and e) are performed simultaneously in a single reaction (p. 8, paragraph 90, where in a preferred embodiment, the fragmentation/nicking and labeling steps are performed at the same time).

With regard to claim 3, Blume teaches an embodiment of claim 1, wherein step d) comprises: generating abasic sites in the second strand cDNA and cleaving the second strand cDNA at the abasic sites (p. 8, paragraph 86, where dUTP is incorporated into cDNA during first or second strand cDNA synthesis, see Figures 1 and 2 and where uracil DNA glycosylase recognizes uracil in DNA and the uracil is removed, followed by treatment with an endonuclease such as endonuclease IV or V, leaving a 3'OH free).

With regard to claim 4, Blume teaches an embodiment of claim 3, wherein the abasic sites are generated by incubating with a uracil DNA glycosylase enzyme (p. 8, paragraph 86, where uracil DNA glycosylase recognizes uracil in DNA and the uracil is removed, followed by treatment with an endonuclease such as endonuclease IV or V, leaving a 3'OH free).

With regard to claim 5, Blume teaches an embodiment of claim 3, wherein the step of cleaving the second strand cDNA at the abasic sites comprises incubating the second strand cDNA with an apurinic endonuclease (p. 8, paragraph 86, where uracil DNA glycosylase recognizes uracil in DNA and the uracil is removed, followed by treatment with an endonuclease such as endonuclease IV or V, leaving a 3'OH free).

With regard to claim 6, Blume teaches an embodiment of claim 5, wherein the apurinic endonuclease is Endonuclease IV (p. 8, paragraph 86, where uracil DNA glycosylase recognizes uracil in DNA and the uracil is removed, followed by treatment with an endonuclease such as endonuclease IV or V, leaving a 3'OH free).

With regard to claim 9, Blume teaches an embodiment of claim 1, wherein the first strand cDNA is synthesized in the presence of an RNA dependent DNA polymerase and second strand cDNA is synthesized in the presence of a DNA dependent DNA polymerase (p. 1, paragraph 12,

where an RNA-dependent DNA polymerase such as reverse transcriptase will synthesize the first strand of cDNA, p. 2, paragraph 18, where the second strand cDNA synthesis is catalyzed by the Klenow fragment).

With regard to claim 12, Blume teaches an embodiment of claim 1, wherein steps d) and e) are performed under isothermal conditions (p. 9, paragraph 104, 112, 121, or 129, where samples were incubated at 37 °C).

With regard to claim 13, Blume teaches an embodiment of claim 1, wherein steps d) and e) are performed at 37°C (p. 9, paragraph 104, 112, 121, or 129, where samples were incubated at 37 °C).

With regard to claim 14, Blume teaches an embodiment of claim 1, wherein the Endonuclease V is used to nick the second strand cDNA in step d) (p. 8, paragraph 86, where uracil DNA glycosylase recognizes uracil in DNA and the uracil is removed, followed by treatment with an endonuclease such as endonuclease IV or V, leaving a 3'OH free).

With regard to claim 15, Blume teaches an embodiment of claim 1, wherein the reaction mixture of step c) further comprises dTTP and the ratio of dTTP to dUTP in the starting mixture is greater than about 5 to 1 (p. 9, paragraph 89, where a preferred embodiment is represented by a ratio of 1 dU to 3 dT).

With regard to claim 16, Blume teaches an embodiment of claim 1, wherein the reaction mixture of step e) further comprises a labeled nucleotide (p. 9, paragraph 105 or 122, where the labeling reaction is described p. 2, paragraph 21, legend to Figure 1, where the free 3' OH is labeled by polymerization with terminal transferase).

With regard to claim 17, Blume teaches an embodiment of claim 16, wherein the labeled nucleotide is biotin-dATP (p. 8, paragraph 83, where the fragments are end labeled using biotinylated DNA labeling reagent).

With regard to claim 18, Blume teaches an embodiment of claim 1, wherein the first strand cDNA is synthesized by a method comprising: hybridizing at least one primer to the nucleic acid sample and extending the primer with a polymerase (Figure 1, where a primer is used to prime first strand cDNA synthesis).

With regard to claim 22, Blume teaches a method of detecting a target sequence in a nucleic acid sample comprising a complex mixture of sequences, comprising:

a) amplifying the nucleic acid sample by the method of claim 1 (p. 8, paragraph 86, as described previously);

b) labeling the nucleic acids in the amplified nucleic acid sample with a detectable label (p. 8, paragraph 83, where the fragments are end labeled using biotinylated DNA labeling reagent);

c) hybridizing the labeled, amplified nucleic acids to an array of probes comprising at least one probe that is perfectly complementary to the target sequence over the length of the probe (p. 9, paragraph 91, where fragments are hybridized to a microarray; p. 142, where fragments are analyzed by hybridization to an array);

d) detecting a hybridization pattern (p. 9, paragraph 91, where fragments are hybridized to a microarray; p. 142, where fragments are analyzed by hybridization to an array); and

e) determining if the target sequence is present or absent based on the hybridization pattern (p. 9, paragraph 91, where fragments are hybridized to a microarray; p. 142, where fragments are analyzed by hybridization to an array).

With regard to claim 23, Blume teaches an embodiment of claim 21, wherein the label is biotin (p. 8, paragraph 83, where the fragments are end labeled using biotinylated DNA labeling reagent).

With regard to claim 24, Blume teaches an embodiment of l, wherein the nucleic acid sample comprises RNA and first strand cDNA is synthesized using an RNA dependent DNA polymerase (p. 1, paragraph 12, where an RNA-dependent DNA polymerase such as reverse transcriptase will synthesize the first strand of cDNA, p. 2, paragraph 18, where the second strand cDNA synthesis is catalyzed by the Klenow fragment).

With regard to claim 31, Blume teaches a method of genotyping at least one polymorphism from a sample comprising:

a) obtaining a nucleic acid sample (p. 8, paragraph 86, where RNA transcript samples are used as templates);

b) contacting the nucleic acid sample with the appropriate reagents to synthesize a first strand cDNA from the nucleic acid sample (p. 8, paragraph 86, where the transcripts are used as templates for reverse transcription);

c) synthesizing a second strand cDNA in a reaction mixture comprising dUTP (p. 8, paragraph 86, where dUTP is incorporated into cDNA during first or second strand cDNA synthesis, see Figures 1 and 2);

c) synthesizing a second strand cDNA in a reaction mixture comprising dUTP (p. 8, paragraph 86, where dUTP is incorporated into cDNA during first or second strand cDNA synthesis, see Figures 1 and 2);

d) nicking the second strand cDNA at one or more positions where dUTP was incorporated to generate one or more nicks in the second strand cDNA (p. 8, paragraph 86, where uracil DNA glycosylase recognizes uracil in DNA and the uracil is removed, followed by treatment with an endonuclease such as endonuclease IV or V, leaving a 3'OH free); and

e) extending the second strand cDNA from the one or more nicks in a reaction mixture comprising dUTP and a DNA polymerase with strand displacing activity, wherein downstream fragments of the second strand cDNA are displaced by the DNA polymerase to generate displaced fragments with a detectable label (p. 9, paragraph 105 or 122, where the labeling reaction is described p. 2, paragraph 21, legend to Figure 1, where the free 3' OH is labeled by polymerization with terminal transferase);

f) labeling the displaced fragments with a detectable label (p. 8, paragraph 83, where the fragments are end labeled using biotinylated DNA labeling reagent);

g) hybridizing the labeled displaced fragments to an array of probes comprising at least one probe that is perfectly complementary to the target sequence over the length of the probe (p. 9, paragraph 91, where fragments are hybridized to a microarray; p. 142, where fragments are analyzed by hybridization to an array);

a) detecting a hybridization pattern (p. 9, paragraph 91, where fragments are hybridized to a microarray; p. 142, where fragments are analyzed by hybridization to an array); and

b) determining if the target sequence is present or absent based on the hybridization pattern (p. 9, paragraph 91, where fragments are hybridized to a microarray; p. 142, where fragments are analyzed by hybridization to an array).

Regarding claims 1 and 31, Blume does not explicitly teach step e) where the nicked strand of cDNA is extended with a DNA polymerase with strand displacing activity, wherein downstream fragments of the second strand cDNA are displaced by the polymerase. Instead, Blume teaches labeling of the free 3'OH with terminal transferase, an enzyme which polymerizes the addition of nucleotides in a template independent manner (p. 8, paragraph 83).

McCarthy teaches the extension of free 3'OH sites using a polymerase with strand displacement activity, for example at p. 6, paragraph 102, where the DNA polymerase synthesizes new DNA from the 3'OH termini and displaces the downstream DNA.

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have modified the technique of cDNA synthesis, fragmentation, and labeling taught by Blume to include the step taught by McCarthy, where the free 3'OH termini are extended using a strand displacing polymerase. As taught by McCarthy, "The method according to the invention provides a means of generating multiple copies of discrete single stranded primers downstream of an initiating primer. This offers exceptional specificity for detection purposes, as the discrete downstream primers can only be generated if the target template nucleic acid is present" (p. 5, paragraph 71). Given the benefit of specificity in detection provided by the method taught by McCarthy, one of ordinary skill in the art at the time the invention was made would have been motivated to substitute the step of extending the free 3'OH using a strand displacing polymerase for the step of labeling using a terminal transferase as taught by Blume with a reasonable expectation for success to achieve improved specificity for detection.

6. Claims 1-10, 12-16, 18-22, 24-28, and 31 are rejected under 35 U.S.C. 103(a) as being unpatentable over Sehgal et al. (Journal of Surgical Oncology, 1998, vol. 67, p. 234-241) in view of Eberwine et al. (PNAS, 1992, vol. 89, p. 3010-3014) and McCarthy et al. (US PgPub 2004/0067559; April 2004). Sehgal teaches a method of differential hybridization of cDNA expression arrays (Abstract).

With regard to claim 1, Sehgal teaches a method of amplification of a nucleic acid sample comprising: a) obtaining a nucleic acid sample (p. 235, col. 2, 'differential hybridization' heading, where total RNA was isolated from glioblastoma multiforme tumor tissue);
b) contacting the nucleic acid sample with the appropriate reagents to synthesize a first strand cDNA (p. 235, col. 2, 'differential hybridization' heading, where oligo dT, random hexamer and CDS primers were incubated with RNA and reverse transcriptase).

With regard to claim 18, Sehgal teaches an embodiment of claim 1, wherein the first strand cDNA is synthesized by a method comprising: hybridizing at least one primer to the nucleic acid sample and extending the primer with a polymerase (p. 235, col. 2, 'differential hybridization' heading, where oligo dT, random hexamer and CDS primers were incubated with RNA and reverse transcriptase).

With regard to claim 19, Sehgal teaches an embodiment of claim 18, wherein the nucleic acid sample comprises DNA and the polymerase is an RNA dependent DNA polymerase (p. 235, col. 2, 'differential hybridization' heading, where oligo dT, random hexamer and CDS primers were incubated with RNA and reverse transcriptase and where reverse transcriptase is an RNA dependent DNA polymerase).

With regard to claim 20, Sehgal teaches an embodiment of claim 18, wherein the at least one primer comprises a 3' oligo dT portion (p. 235, col. 2, 'differential hybridization' heading, where oligo dT, random hexamer and CDS primers were incubated with RNA and reverse transcriptase).

With regard to claim 21, Sehgal teaches an embodiment of claim 18, wherein the at least one primer comprises a mixture of primers of random sequence wherein the primers are of a common length and the length is between 6 and 15 nucleotides (p. 235, col. 2, 'differential hybridization' heading, where oligo dT, random hexamer and CDS primers were incubated with RNA and reverse transcriptase and where a random hexamer would have a length of 6 nucleotides).

With regard to claim 22, Sehgal teaches a method of detecting a target sequence in a nucleic acid sample comprising a complex mixture of sequences, comprising:
a) amplifying the nucleic acid sample by the method of claim 1 (p. 235, col. 2, 'differential hybridization' heading, where total RNA was isolated from glioblastoma multiforme tumor tissue and as described more fully above);
b) labeling the nucleic acids in the amplified nucleic acid sample with a detectable label (p.235, col. 2, 'differential hybridization' heading, where the cDNA was labeled with 40 μCi dATP^{32});
c) hybridizing the labeled, amplified nucleic acids to an array of probes comprising at least one probe that is perfectly complementary to the target sequence over the length of the probe (p. 235, col. 2, 'differential hybridization' heading, where cDNAs were hybridized to two human cDNA expression arrays in separate bags);

d) detecting a hybridization pattern (p. 236, col. 1, where the hybridized arrays were exposed to X-ray film); and

e) determining if the target sequence is present or absent based on the hybridization pattern (Figures 1-2).

With regard to claim 24, Sehgal teaches an embodiment of l, wherein the nucleic acid sample comprises RNA and first strand cDNA is synthesized using an RNA dependent DNA polymerase (p. 235, col. 2, 'differential hybridization' heading, where oligo dT, random hexamer and CDS primers were incubated with RNA and reverse transcriptase and where reverse transcriptase is an RNA dependent DNA polymerase).

With regard to claim 25, Sehgal teaches an embodiment of claim 20, wherein first strand cDNA is synthesized by a primer comprising oligo dT (p. 235, col. 2, 'differential hybridization' heading, where oligo dT, random hexamer and CDS primers were incubated with RNA and reverse transcriptase).

With regard to claims 26 and 28, Sehgal teaches an embodiment of claim 20 or 23, wherein first strand cDNA synthesis is primed by a plurality of locus specific primers (p. 236, col. 2, 'gene specific primers' heading).

With regard to claim 31, Sehgal teaches a method of genotyping at least one polymorphism from a sample comprising:

a) obtaining a nucleic acid sample (p. 235, col. 2, 'differential hybridization' heading, where total RNA was isolated from glioblastoma multiforme tumor tissue);

b) contacting the nucleic acid sample with the appropriate reagents to synthesize a first strand cDNA from the nucleic acid sample (p. 235, col. 2, 'differential hybridization' heading, where

oligo dT, random hexamer and CDS primers were incubated with RNA and reverse transcriptase);

f) labeling the displaced fragments with a detectable label (p.235, col. 2, 'differential hybridization' heading, where the cDNA was labeled with 40 μCi dATP^{32});

g) hybridizing the labeled displaced fragments to an array of probes comprising at least one probe that is perfectly complementary to the target sequence over the length of the probe (p. 235, col. 2, 'differential hybridization' heading, where cDNAs were hybridized to two human cDNA expression arrays in separate bags);

a) detecting a hybridization pattern (p. 236, col. 1, where the hybridized arrays were exposed to X-ray film); and

b) determining if the target sequence is present or absent based on the hybridization pattern (Figures 1-2).

Regarding claims 1 and 31, Sehgal does not explicitly teach the step c) where a second strand of cDNA is synthesized and instead teaches that the first strand cDNA was labeled with a random primer. Eberwine explicitly teaches steps directed to first and second strand cDNA synthesis, followed by further steps directed to the in vitro transcription of the cDNA to generate amplified RNA.

With regard to claims 1 and 31, Eberwine teaches a step in cDNA synthesis where second strand cDNA is synthesized in a reaction mixture (Figure 2, where second strand cDNA is synthesized; p. 3011, col. 2, 'amplification and reamplification' heading, where second strand cDNA synthesis was accomplished using T4 and Klenow).

With regard to claim 9, Eberwine teaches an embodiment of claim 1, wherein the first strand cDNA is synthesized in the presence of an RNA dependent DNA polymerase and second strand cDNA is synthesized in the presence of a DNA dependent DNA polymerase (Figure 2, where second strand cDNA is synthesized; p. 3011, col. 2, 'amplification and reamplification' heading, where second strand cDNA synthesis was accomplished using T4 and Klenow).

Regarding claims 1 and 31, neither Sehgal or Eberwine teach steps d) and e) comprising steps directed to synthesizing the second strand of cDNA in a reaction comprising dUTP, nicking the second strand cDNA at one or more positions where dUTP was incorporated and extending the second strand cDNA from one or more nicks in the mixture using a strand displacing DNA polymerase.

McCarthy teaches a method for amplification of a template nucleic acid through primer extension, cleavage of modified nucleotides, and extending the 3'-OH generated by the cleavage (Abstract). Regarding claims 1 and 31, McCarthy teaches steps c) to e) as follows:
c) synthesizing a second strand cDNA in a reaction mixture comprising dUTP (p. 3, paragraphs 32-39, where a primer is extended in the presence of at least one modified DNA precursor which is a substrate for a DNA glycosylase; p. 4, paragraphs 49-55, where the modified DNA precursor is dUTP; p. 6, paragraph 91-102; see also p. 13-14, example 1, paragraph 167-168, where the reaction included 0.2 mM each of dATP, dGTP, dUTP and 0.02 mM dCTP and α^{32}PdCTP);
d) nicking the second strand cDNA at one or more portions where dUTP was incorporated to generate one or more nicks (p. 4, paragraphs 49-59, where the modified dUTP is excised by uracil DNA-glycosylase (UDG) and where the cleavage of the abasic site is by Endonuclease IV; p. 6, paragraphs 91-102; see also p. 13-14, example 1, paragraph 168, where the reaction was

dropped to 37°C and 5 units of Klenow fragment (3'-5' exo-), 1 unit of E. coli Uracil DNA glycosylase were added along with 2 units of Endonuclease IV); and

e) extending the second strand cDNA from the one or more nicks in a reaction mixture comprising dUTP and a DNA polymerase with a strand displacing activity, wherein downstream fragments of the second strand cDNA are displaced (p. 6, paragraph 102, where the DNA polymerase synthesizes new DNA from the 3'OH termini and displaces the downstream DNA; p. 3, paragraph 32-39, where the free 3;OH generated by the cleavage at the abasic site is extendable by the DNA polymerase; see also p. 13-14, example 1, paragraph 168-169).

With regard to claim 2, McCarthy teaches an embodiment of claim 1 wherein steps d) and e) are performed simultaneously in a single reaction (p. 6, paragraph 96, where it is noted that the steps cycle continuously until one of the reagents becomes limiting; p. 6, paragraph 98, where the components of the reaction are all simultaneously present in the same reaction and a continuous cycle of extension and cleavage results in the amplification of multiple copies of displaced downstream fragments).

With regard to claim 3, McCarthy teaches an embodiment of claim 1, wherein step d) comprises: generating abasic sites in the second strand cDNA and cleaving the second strand cDNA at the abasic sites (p. 4, paragraphs 49-59, where the modified dUTP is excised by uracil DNA-glycosylase (UDG) and where the cleavage of the abasic site is by Endonuclease IV).

With regard to claim 4, McCarthy teaches an embodiment of claim 3, wherein the abasic sites are generated by incubating with a uracil DNA glycosylase enzyme (p. 4, paragraphs 49-59, where the modified dUTP is excised by uracil DNA-glycosylase (UDG) and where the cleavage of the abasic site is by Endonuclease IV).

With regard to claim 5, McCarthy teaches an embodiment of claim 3, wherein the step of cleaving the second strand cDNA at the abasic sites comprises incubating the second strand cDNA with an apurinic endonuclease (p. 4, paragraphs 49-59, where the modified dUTP is excised by uracil DNA-glycosylase (UDG) and where the cleavage of the abasic site is by Endonuclease IV).

With regard to claim 6, McCarthy teaches an embodiment of claim 5, wherein the apurinic endonuclease is Endonuclease IV (p. 4, paragraphs 49-59, where the modified dUTP is excised by uracil DNA-glycosylase (UDG) and where the cleavage of the abasic site is by Endonuclease IV).

With regard to claim 7, McCarthy teaches an embodiment of claim 3, wherein the step of cleaving the second strand cDNA at abasic sites comprises incubating the second strand cDNA at high temperatures (p. 4, paragraph 59, where agents which cleave 3' to the phosphate moiety to generate a 3' terminus which a 3'-P group are heat, alkali and DNA repair enzymes).

With regard to claim 8, McCarthy teaches an embodiment of claim 3, wherein the step of cleaving the second strand cDNA at abasic sites comprises incubating the second strand cDNA under alkaline conditions (p. 4, paragraph 59, where agents which cleave 3' to the phosphate moiety to generate a 3' terminus which a 3'-P group are heat, alkali and DNA repair enzymes).

With regard to claim 10, McCarthy teaches an embodiment of claim 1, wherein the strand displacing DNA polymerase is selected from the group consisting of the Klenow fragment, Bst and phi29 (p. 13-14, example 1, paragraph 168, where the reaction was dropped to 37oC and 5 units of Klenow fragment (3'-5' exo-), 1 unit of E. coli Uracil DNA glycosylase were added along with 2 units of Endonuclease IV).

With regard to claim 12, McCarthy teaches an embodiment of claim 1, wherein steps d) and e) are performed under isothermal conditions (p. 13-14, example 1, paragraph 168, where the reaction was dropped to 37°C and 5 units of Klenow fragment (3'-5' exo-), 1 unit of E. coli Uracil DNA glycosylase were added along with 2 units of Endonuclease IV, where the reaction was carried out at 37°C).

With regard to claim 13, McCarthy teaches an embodiment of claim 1, wherein steps d) and e) are performed at 37°C (p. 13-14, example 1, paragraph 168, where the reaction was dropped to 37°C and 5 units of Klenow fragment (3'-5' exo-), 1 unit of E. coli Uracil DNA glycosylase were added along with 2 units of Endonuclease IV, where the reaction was carried out at 37°C).

With regard to claim 14, McCarthy teaches an embodiment of claim 1, wherein the Endonuclease V is used to nick the second strand cDNA in step d) (p. 4, paragraph 62, where it is noted that the 3' endonuclease may be Endonuclease V).

With regard to claim 15, McCarthy teaches an embodiment of claim 1, wherein the reaction mixture of step c) further comprises dTTP and the ratio of dTTP to dUTP in the starting mixture is greater than about 5 to 1 (p. 10-11, paragraph 138-139, where dTTP is included with a ratio of dUTP to generate fragments of multiple sizes).

With regard to claim 16, McCarthy teaches an embodiment of claim 1, wherein the reaction mixture of step e) further comprises a labeled nucleotide (p. 13-14, example 1, paragraph 167-168, where the reaction included 0.2 mM each of dATP, dGTP, dUTP and 0.02 mM dCTP and α^{32}PdCTP).

With regard to claim 27, McCarthy teaches an embodiment of claim 1, wherein the nucleic acid comprises genomic DNA (p. 9-10, paragraph 133-134, where genomic DNA can serve as the template for the GMA reaction).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to incorporate the method of generation of abasic sites in a nucleic acid sample, followed by amplification of the displaced downstream fragments through cycling of the primer extension, cleavage and extension process described above into a method of cDNA synthesis as disclosed by Sehgal and Eberwine, methods which are well known in the prior art at the time the invention was made. McCarthy explicitly teaches "When an RNA template is used, a DNA polymerase which can utilize an RNA template is required, typically such an enzyme is reverse transcriptase" (p. 3, paragraph 47), providing a direct teaching that the method can be applied to RNA targets as part of reverse transcription. Furthermore, the method disclosed by McCarthy "provides a means of generating multiple copies of discrete single stranded primers downstream of an initiating primer. This offers exceptional specificity for detection purposes, as the discrete downstream primers can only be generated if the target template nucleic acid is present" (p. 5, paragraph 71)". McCarthy also notes that "the method according to the invention has significant advantages over existing technologies in that it is more versatile and more flexible with respect to providing a single high throughput process that can be easily adapted to multiple different formats in the fields of DNA detection, quantitation and characterization" (p. 3, paragraph 37). Therefore, given the benefit of specificity in detection provided by the method taught by McCarthy, one of ordinary skill in the art at the time the invention was made would have been motivated to apply the method of glycosylase mediated amplification taught by

McCarthy to a method of cDNA synthesis, as exemplified by Sehgal with a reasonable expectation for success.

7. Claims 10-11 are rejected under 35 U.S.C. 103(a) as being unpatentable over Sehgal et al. (Journal of Surgical Oncology, 1998, vol. 67, p. 234-241) in view of Eberwine et al. (PNAS, 1992, vol. 89, p. 3010-3014) and McCarthy et al. (US PgPub 2004/0067559; April 2004) as applied to claims 1-10, 12-16, 18-22, 24-28, and 31 above, and further in view of Blanco et al. (US Patent 5,198,543; March 1993). Sehgal teaches a method of differential hybridization of cDNA expression arrays (Abstract).

Sehgal in view of Eberwine and McCarthy teach the limitations of claims 1-10, 12-16, 18-22, 24-28, and 31 as recited in the 103 above. However, Sehgal, Eberwine and McCarthy do not teach the use of a strand displacing DNA polymerase phi29 with decreased exonuclease activity. Blanco teaches an improved method for determining the nucleotide sequence of a DNA molecule (Abstract).

With regard to claim 10, Blanco teaches an embodiment of claim 1, wherein the strand displacing DNA polymerase is selected from the group consisting of the Klenow fragment, Bst and phi29 (Abstract, where the improvement is a modification of the phi29 DNA polymerase).

With regard to claim 11, Blanco teaches an embodiment of claim 1, wherein the DNA polymerase is a phi29 variant that has reduced exonuclease activity (col. 2, lines 43-49, where a phi29 modified to reduce exonuclease activity to less than 10% of naturally occurring phi29 polymerase is incorporated into the method).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to incorporate the phi29 polymerase enzyme with reduced exonuclease activity as taught by Blanco into the method of modification of cDNA synthesis using the digestion/nicking method taught by McCarthy with a reasonable expectation for success. As noted by Blanco, "the invention provides a polymerase which is highly processive, and may be produced with a low exonuclease activity. The high processivity of the polymerase makes it suitable, not only for DNA sequencing, but also for amplification of very large fragments of DNA (in excess of 10 kilobases in length). This makes the polymerase useful in a polymerase chain reaction (PCR) type procedure or in replicative type, protein primed, extension reactions" (col. 3, lines 50-61). One of ordinary skill in the art at the time the invention was made would have recognized the benefit of improved read-length provided by the modified phi29 polymerase and would have been motivated to substitute this strand displacing enzyme for the Klenow fragment taught by Eberwine and McCarthy for extension of the 3'OH termini which are crucial for the practice of the invention, and such modification would have a reasonable expectation for success.

8. Claims 17-23 are rejected under 35 U.S.C. 103(a) as being unpatentable over Sehgal et al. (Journal of Surgical Oncology, 1998, vol. 67, p. 234-241) in view of Eberwine et al. (PNAS, 1992, vol. 89, p. 3010-3014) and McCarthy et al. (US PgPub 2004/0067559; April 2004) as applied to claims 1-10, 12-16, 18-22, 24-28, and 31 above, and further in view of Wang et al. (US Patent 6,004,755; December 1999). Sehgal teaches a method of differential hybridization of cDNA expression arrays (Abstract).

Sehgal in view of Eberwine and McCarthy teach the limitations of claims 1-10, 12-16, 18-22, 24-28, and 31 as recited in the 103 rejection stated above. However, neither Sehgal, Eberwine or McCarthy teach the inclusion of biotin as a detectable label.

Wang teaches the inclusion of biotin in cDNA synthesis as part of quantitative gene expression analysis (Abstract).

With regard to claim 17, Wang teaches an embodiment of claim 16, wherein the labeled nucleotide is biotin-dATP (col. 9, lines 20-28, where the labeled nucleotide is biotin).

With regard to claim 23, Wang teaches an embodiment of claim 21, wherein the label is biotin (col. 9, lines 20-28, where the labeled nucleotide is biotin).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to apply the incorporation of biotin modified nucleotides into the cDNA synthesis technique taught by Sehgal and Eberwine and the method of fragmentation and amplification taught by McCarthy. The inclusion of a detectable label allows for specific detection of the hybridization pattern on the array and specifically when biotin is used as the label, "one contacts the array with streptavidin-fluorescer conjugate under conditions sufficient for binding between the specific binding member pairs to occur. Following contact, any unbound members of the signal producing system will then be removed, e.g., by washing" (col. 7, lines 11-22). Recognizing the specificity provided by the inclusion of a detectable label, one of ordinary skill in the art at the time the invention was made would have been motivated to incorporate a biotin label into the method taught by Sehgal, Eberwine and McCarthy with a reasonable expectation for success.

9. Claims 29-30 are rejected under 35 U.S.C. 103(a) as being unpatentable over Sehgal et al. (Journal of Surgical Oncology, 1998, vol. 67, p. 234-241) in view of Eberwine et al. (PNAS, 1992, vol. 89, p. 3010-3014) and McCarthy et al. (US PgPub 2004/0067559; April 2004) as applied to claims 1-9, 12-16, 18-22, 24-28, and 31 above, and further in view of Caskey et al. (US Patent 5,364,759; November 1994). Sehgal teaches a method of differential hybridization of cDNA expression arrays (Abstract).

Sehgal in view of Eberwine and McCarthy teach the limitations of claims 1-10, 12-16, 18-22, 24-28, and 31 as recited in the 103 rejection stated above. However, neither Sehgal, Eberwine or McCarthy teach the inclusion of biotin as a detectable label.

With regard to claim 29, Caskey teaches an embodiment of claim 1, wherein the nucleic acid sample comprises adaptor ligated DNA fragments (col. 10, example 3, where a linker of non-complementary DNA is ligated to the population of blunt-ended molecules).

With regard to claim 30, Caskey teaches an embodiment of claim 1, wherein the nucleic acid sample comprises adaptor ligated DNA fragments that have been amplified by PCR (col. 10, example 3, where a linker of non-complementary DNA is ligated to the population of blunt-ended molecules and the samples are amplified by PCR).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have incorporated the adaptor ligated PCR amplification fragments disclosed by Caskey into the method of DNA detection and characterization disclosed by Sehgal, Eberwine and McCarthy. As noted by Caskey, "this assay incorporates internal or external standards, provides higher sensitivity, requires shorter analysis time, lowers expense, and enables precise identification of alleles". Therefore, given the benefit of a more rapid method, with

greater precision and sensitivity one of ordinary skill in the art would have been motivated to include the adaptor ligation method taught by Caskey as a target nucleic acid for the method taught by Sehgal, Eberwine and McCarthy with a reasonable expectation for success.

10. Claim 32 is rejected under 35 U.S.C. 103(a) as being unpatentable over Sehgal et al. (Journal of Surgical Oncology, 1998, vol. 67, p. 234-241) in view of Eberwine et al. (PNAS, 1992, vol. 89, p. 3010-3014) and McCarthy et al. (US PgPub 2004/0067559; April 2004) as applied to claims 1-9, 12-16, 18-22, 24-28, and 31 above, and further in view of Lipshutz et al. (US Patent 6,300,063; October 2001).

With regard to claim 32, Lipshutz teaches an embodiment of claim 31, wherein the array of probes comprises a plurality of genotyping probe sets wherein each probe set comprises a first probe that is perfectly complementary to a first allele of a SNP and a second probe that is perfectly complementary to a second allele of the SNP wherein the first and second probes are between 20 and 50 nucleotides in length and the central position of each probe is complementary to the polymorphic position of the SNP (Figure 2B, where 20mer oligonucleotides are designed which incorporate substitutions 7, 10 and 13 bp from the 3' end in order to interrogate a polymorphic site within an oligonucleotide; see for example, col. 2, lines 17-30).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have incorporated the specific type of array disclosed by Lipshutz into the array hybridization taught by Sehgal. As noted by Lipshutz, "the present invention generally provides rapid and efficient methods for screening samples of genomic material for polymorphisms and arrays specifically designed for carrying out these analyses" (col. 4, lines 20-

25). Lipshutz goes on to note "The probes are typically arranged in detection blocks, each block being capable of discriminating the three genotypes for a given marker, e.g., the heterozygote or either of the two homozygotes. The method allows for rapid, automatable analysis of genetic linkage to even complex polygenic traits" (col. 4, lines 30-35).Therefore, given the benefit of a rapid and automatable analysis platform capable of discriminating polymorphisms in complex polygenic traits one of ordinary skill in the art would have been motivated to include the array taught by Lipshutz into the method of genetic analysis taught by Sehgal, Eberwine and McCarthy with a reasonable expectation for success.

Conclusion

11. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. Porat et al. (US PgPub 2004/0166493; August 2004) teaches a method for identifying variations, such as single nucleotide polymorphisms (Abstract).

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Stephanie K. Mummert whose telephone number is 571-272-8503. The examiner can normally be reached on M-F, 8:30-5.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Stephanie K. Mummert
Examiner
Art Unit 1637

SKM

KENNETH R. HORLICK, PH.D
PRIMARY EXAMINER

4/13/06